REMARKS/ARGUMENTS

Claim rejections under 35 U.S.C. §102(b):

The Examiner has maintained her rejection of claims 1, 3, and 5 under 35 U.S.C. §102(b) as being anticipated by Greathouse, et al. (U.S. Pat. No. 3,023,144) and by Chastain, et al. (U.S. Pat. No. 5,153,229). For the following reasons, the Applicant respectfully traverses this rejection.

The Examiner argues that Greathouse et al. teaches d-limonene having "low activity but does not state it (limonene) has no activity." (Examiner's Action, page 2). The Examiner further points out that the Greathouse reference teaches syngergistic activity when d-limonene is combined with other derivatives, further supporting the Examiner's argument that "d-limonene played an active part in the fungicidal and bacterialcidal action . . . ." (Examiner's Action, page 3). The Applicant respectfully disagrees with the Examiner's characterization of this reference, for on the contrary, Greathouse, et al. does not teach any therapeutically effective germicidal activity on the part of d-limonene, but instead states that "d-limonene per se has been found to possess comparatively little germicidal activity." (Col. 1, lines 62-62). And the fact that limonene, due to its activity as a solvent and penetrant, may enhance the bactericidal activity of true antibacterial agents, does not equate to limonene by itself being an effective bacteriocidal. Applicant's Claim 1 provides for the application of a formulation comprising d-limonene to the skin the nasal cavity of an animal "for a time sufficient for said d-limonene to effectively eradicate or inhibit the growth of said bacteria." Greathouse does not teach the use of d-limonene as the compound responsible for this antibacterial activity.

In view of the foregoing, it is respectfully submitted that claim 1 as amended is not anticipated by Greathouse et al. or Chastain et al.

Claim rejections under 35 U.S.C. §102(e):

The Examiner has also maintained her rejection of claims 1, 3, and 5 under 35 U.S.C. §102(e) as being anticipated by Franklin (U.S. Pub. No. 2003/0180349). For the following reasons, the Applicant respectfully traverses this rejection.

It is respectfully submitted that Franklin is an improper prior art reference with respect to the Applicant's claims. Franklin has a filing date of December 9, 2002, and claims the benefit of the filing date (December 7, 2001) of an earlier filed provisional application. The Applicant has obtained a copy of Franklin's provisional application (copy attached hereto as Ex. A), and no where is there any teaching whatsoever of the use of d-limonene as an antibacterial agent. Consequently, with respect to any teachings of the use of limonene as an antibacterial agent, the

Franklin reference is only entitled to an effective date of December 9, 2002 as a prior art reference. The Applicant's application, while filed on July 7, 2003, has an effective priority date of July 8, 2002 (SN 60/394,333), and which clearly supports the Applicant's currently pending claims and pre-dates the effective prior date of the Franklin reference (i.e. December 9, 2002) by approximately five months. In view of the foregoing, t is the Examiner's burden to show where there is a disclosure or teaching of the use of d-limonene as an antibacterial agent in the in the Franklin provisional application. Absent such a teaching, the Applicant respectfully requests that the Franklin published application be withdrawn as a prior art reference against the Applicant's claims.

Conclusion:

In view of the foregoing amendments and remarks, it is respectfully submitted that claim 1 as well as claims 2-5 dependent thereon, are patentable in view of the cited art, and thus withdrawal of the Examiner's rejections is hereby requested.

Respectfully submitted,

Laura G. Barrow (Reg. No. 35,437)

Date: 12/26/06

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CERTIFICATE UNDER 37 CFR 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on December 26, 2006.

Laura G. Barrow

Class	Subclass	ISSUE CLASSIFICATION

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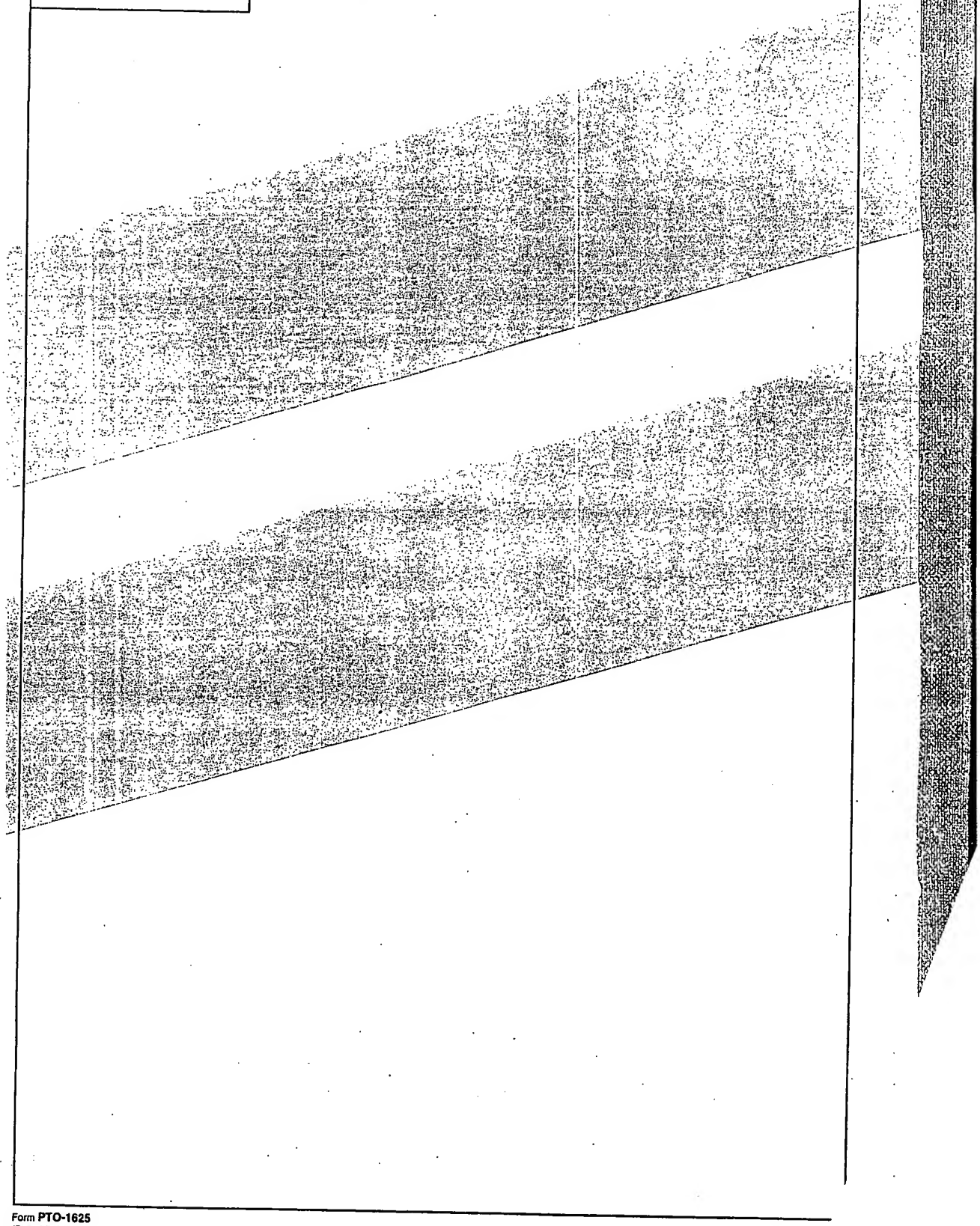
PROVISIONAL APPLICATION NUMBER

Ex. A



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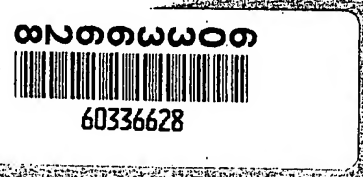

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CONTENTS

Date Entered or Counted			Date Received or Mailed
	1.	Application papers	12-5-01
	2.	Royal L. Cox	
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	4.		
	5.		
	6.		
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	32.		

POSITION	INITIALS	ID NO.	DATE
FEE DETERMINATION			
O.I.P.E. CLASSIFIER			
FORMALITY REVIEW	A	676	04/06/02
RESPONSE FORMALITY REVIEW			

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
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FEE RECORD SHEET

PTO-1556
(5/87)

*U.S. GPO: 2000-468-987/39595

PTO/SB/16 (10-01) A/Prov

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
LANNY UDELL	FRANKLIN	5170 CHEMIN DE VIE ATLANTA, GA 30342

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

RESPIRATORY INFECTIONS PREVENTION AND TREATMENT

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number _____ → *Place Customer Number Bar Code Label here*

OR *Type Customer Number here*

☒ Firm or Individual Name	LANNY U. FRANKLIN				
Address	5170 CHEMIN DE VIE				
Address					
City	ATLANTA	State	GA	ZIP	30342
Country	USA	Telephone	404.822.8906	Fax	404.255.0977

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 13 ☐ CD(s), Number

☐ Drawing(s) *Number of Sheets* ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☒ A check or money order is enclosed to cover the filing fees

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☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): 80

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE LUFranklin Date 10.26.01

TYPED or PRINTED NAME LANNY U. FRANKLIN REGISTRATION NO. *(if appropriate)*

TELEPHONE 404.822.8906 Docket Number:

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This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

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TOTAL AMOUNT OF PAYMENT ($) 80

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	LANNY UDELL FRANKLIN
Examiner Name	
Group Art Unit	
Attorney Docket No.	

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   Deposit Account Number
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   ☐ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17
   ☐ Applicant claims small entity status. See 37 CFR 1.27
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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	740	201	370	Utility filing fee	
106	330	206	165	Design filing fee	
107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	80

SUBTOTAL (1) ($) 80

2. EXTRA CLAIM FEES

	Extra Claims		Fee from below	Fee Paid
Total Claims		-20** =	X	=
Independent Claims		- 3** =	X	=
Multiple Dependent				=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claim, if not paid
109	84	209	42	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

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SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	LANNY U. FRANKLIN	Registration No. (Attorney/Agent)		Telephone	404.822.8906
Signature	LUFranklin			Date	10-26-01

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TITLE OF THE INVENTION

RESPIRATORY INFECTIONS PREVENTION AND TREATMENT

BACKGROUND OF THE INVENTION

Field of the Invention

A method of prevention and treatment of respiratory infections by the inhalation of an aerosol solution containing a single bioactive terpene, a bioactive terpene mixture or a liposome-terpene(s) composition with or without a surfactant.

Discussion of the background

As civilization progresses there has been a tendency to stay longer in closed and confined spaces due to work requirements or for physical comfort. This has resulted in an increase in respiratory problems, especially allergies due to the presence of bacteria, fungi and parasites in these confined places. One of the respiratory problems is sinusitis.

Sinusitis is caused by bacteria (streptococci, staphylococci, pneumococci, Haemophilus influenza), viruses (rhinovirus, influenza virus, parainfluenza virus); and fungi (Aspergillus, Dematiaceae, Mucoraceae, Penicillium sp.)

There are four categories of fungal infections affecting the paranasal sinuses. They are acute or fulminant invasive fungal sinusitis, chronic or indolent invasive fungal sinusitis, fungus ball (mycetoma) and allergic fungal sinusitis. The offending fungi originate from the classes Zygomycetes (Mucor spp.) and Ascomycetes (Aspergillus spp). Chronic sinusitis can develop into granulomatous chronic infection that may extend beyond the sinus walls. Allergic fungal sinusitis colonizes the sinuses of an atopic immunocompetent patient and acts as an allergen, eliciting an immune response. Fungal induced sinusitis most often is seen in immunosuppressed individuals such as those with AIDS, leukemia, lymphoma or multiple myeloma, or in people with poor diabetes control. The Mayo Clinic Proceedings shows a report where 96% out of 210 patients with sinusitis had fungi. Chronic sinusitis last more than 30 days is typically identified by symptoms of a drippy nose, congestion, headaches and reduced sense of smell. Fungal sinusitis is known as eosinophilic fungal rhinosinusitis (EFRS) or eosinophilic mucinous rhinosinusitis (EMRS).

Aspergillus may cause several different illnesses, including both infections and allergy. These fungi may lodge in the airways or a distant part of the lung and grow until they form a compact sphere known as a "fungus ball." In people with lung damage or serious underlying illnesses, *Aspergillus* may grasp the opportunity to invade the lungs or the whole body. In some individuals, exposure to these fungi also can lead to asthma or to a lung disease resembling severe inflammatory asthma called allergic bronchopulmonary aspergillosis. This latter condition, which occurs only in a minority of people with asthma, is characterized by wheezing, low-grade fever, and coughing up of brown-flecked masses or mucus plugs. Skin testing, blood tests, X-rays, and examination of the sputum for fungi can help establish the diagnosis. Corticosteroid drugs are usually effective in treating this reaction. Immunotherapy is not helpful.

Terpenes are widespread in nature, mainly in plants as constituents of essential oils. Their building block is the hydrocarbon isoprene $(C_5H_8)_n$. Terpenes have been found to be effective and nontoxic dietary antitumor agents which act through a variety of mechanisms of action (Crowell and Gould, 1994 and Crowell et al, 1996). Terpenes, i.e. geraniol, tocotrienol, perillyl alcohol, b-ionone and d-limonene, suppress hepatic HMG-COA reductase activity, a rate limiting step in cholesterol synthesis, and modestly lower cholesterol levels in animals (Elson and Yu, 1994). D-limonene and geraniol reduced mammary tumors (Elegbede et al, 1984 and 1986 and Karlson et al, 1996) and suppressed the growth of transplanted tumors (Yu et al, 1995).

Terpenes have also been found to inhibit the in-vitro growth of bacteria and fungi (Chaumont and Leger, 1992, Moleyar and Narasimham, 1992 and Pattnaik, et al 1997) and some internal and external parasites (Hooser, et al, 1986). Geraniol was found to inhibit growth of *Candida albicans* and *Saccharomyces cerevisiae* strains by enhancing the rate of potassium leakage and disrupting membrane fluidity (Bard, et al, 1988). B-ionone has antifungal activity which was determined by inhibition of spore germination, and growth inhibition in agar (Mikhlin et al, 1983 and Salt et al, 1986). Teprenone (geranylgeranylacetone) has an antibacterial effect on H. pylori (Ishii, 1993). Solutions of 11 different terpenes were effective in inhibiting the growth of pathogenic bacteria in in-vitro tests; levels ranging between 100 ppm and 1000 ppm were effective. The terpenes were diluted in water with 1% polysorbate 20 (Kim et al, 1995). Diterpenes, i.e. trichorabdal A (from R. Trichocarpa) has shown a very strong antibacterial effect against H. pylori (Kadota, et al, 1997).

Rosanol a commercial product with 1% rose oil has been shown to inhibit the growth of several bacteria (*Pseudomona, Staphylococus, E. coli and H pylori*). Geraniol is the active component (75%) of rose oil. Rose oil and geraniol at a concentration of 2 mg/lt. inhibited the growth of H pylori in vitro. Some extracts from herbal medicines have been shown to have an inhibitory effect in H pylori, the most effective being decursinol angelate, decursin, magnolol, berberine, cinnamic acid, decursinol and gallic acid (Bae, et al 1998). Extracts from cashew apple, anacardic acid and (E)-2-hexenal, have shown bactericidal effect against h pylori. There may be different modes of action of terpenes against microorganism; they could (1) interfere with the phospholipid bilayer of the cell membrane (2) impair a variety of enzyme systems (HMG-reductase) and (3) destroy or inactivate genetic material.

SUMMARY OF THE INVENTION

A method of prevention and treatment of respiratory infections by the inhalation of an aerosol solution contain a single bioactive terpene, a bioactive terpene mixture or a liposome-terpene(s) composition.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Poor air quality is one of the major factors that produce respiratory infections in humans. The presence of microorganisms, toxins and allergens is mainly due to poor ventilation, excess moisture and improper cleaning and disinfection. The present invention has the capacity of reducing the incidences of and treating respiratory infections. This invention is comprised of naturally occurring chemicals that are found in plants and that are generally recognized as safe (GRAS) by the FDA. One of the most important aspects of this invention is that due to the mechanism of action terpenes do not generate microbial resistance. There are antimicrobial products containing terpenes, basically in the form of essential oils, but we have found that not all components of the essential oils are biocides. Another advantage of the present invention is that by varying the concentration of terpenes we can have different specificity and biocidal effect and that combining two or more terpenes in the same solution a synergistic effect can be obtained. Another important property of this invention is that the terpenes and surfactant used are generally recognized as safe (GRAS) by the FDA. Another property of this invention is that we can tailor the formulation and obtain biocidal effect over a single type microorganism or change

the formulation and eliminate all types of microorganisms. Applying one of the formulations of the present invention in spray form into the nasal cavities reduces the amount of microorganism responsible of infections like aspergillius and stachybotrys (fungi). These microorganisms are responsible of the majority of respiratory infections present in immuno-deficient patients and children. Several formulations can be obtained by utilizing biocidal terpenes without departing from the principle of the present inventions. Formulations can vary not only in the concentration of terpenes but also in the type of surfactant used. This invention can be readily be mixed with other types of nasal delivery medications. Another advantage of the present invention is that the terpenes present in the formulation can reach all areas of the respiratory system including the lungs. We have found that higher concentrations of certain terpenes can be irritating to the nasal passages, and that by reducing or eliminating these terpenes in the formulation we still have the benefit of the other terpenes. The terpenes we have used in the present invention include citral, carvone, eugenol, b-ionone. All of them have biocidal properties; and other biocidal terpenes can be utilized without departing from the scope of the present invention.

We have observed that the terpenes used in this invention can be targeted to different microorganisms and parasite, we have been able to prove the effectiveness of the present invention against bacteria, fungi and parasites that are of importance for humans and animals. Also the effective terpene dose varies depending on the organism we are interested in eliminating. This invention can be modified in several ways by adding or deleting from the formulation the type of terpene and surfactant. The surfactant can be non-ionic, cationic or anionic.

It will be apparent for those skilled in the art that the aforementioned objects and other advantages may be further achieved by the practice of the present invention.

EXAMPLE 1: Preparation of the terpene mixture

The terpene, terpene mixture or liposome-terpene(s) combination consists of a blend of generally recognized as safe (GRAS) terpenes with a GRAS surfactant. The ratio of terpenes is from 1-99% and the surfactant ratio from 1-99% of the mixture. The terpenes, comprised of natural or synthetic terpenes, are citral, b-ionone, eugenol, geraniol, carvone, terpeniol, carvacrol, anethole or other terpenes with similar properties. The surfactant is preferably polysorbate-80 or other suitable GRAS surfactants.

EXAMPLE 2: Preparation of the terpene mixture

The terpene-water solution is formulated without a surfactant. 100 ppm to 2000 ppm of natural or synthetic terpenes such as citral, b-ionone, geraniol, carvone, terpeniol, carvacrol, anethole, or other terpenes with similar properties are added to water and subjected to a high-shear blending action that forces the terpene(s) into a true solution. The maximum level of terpene(s) that can be solubilized varies with each terpene.

EXAMPLE 3: In in-vitro effectiveness of terpenes against *Aspergillius fumigatus*

This example demonstrates the effectiveness of the terpene mixture against *Aspergillius fumigatus*. *A. fumigatus* was grown for 48 hours at 25-27 °C in tryptose broth and adjusted to approximately 10^5 organisms/ml with sterile saline. For the broth dilution test, terpene mixture containing citral, b-ionone, carvone, eugenol and surfactant was diluted in sterile tryptose broth to give the following dilutions: 1:500, 1:1000, 1:2000, 1:4000, 1:8000, 1:16,000, 1:32,000, 1:64,000 and 1:128,000. Each dilution was added to sterile tubes in 5-ml amounts. Three replicates of each series of dilutions were used. One half ml of the organism was added to each series and incubated at 25-77 °C for 72 hours. After incubation the tubes were observed for growth and plated onto blood agar. The tubes were incubated an additional 24 hours and observed again. The minimum inhibitory concentration for each test organism was determined as the highest dilution that completely inhibits the organism.

Table 1: Results of the inhibitory activity of different dilutions

Organism	Visual assessment of growth *			Growth after subculture to agar plates *			Mean inhibitory dilution
	1	2	3	1	2	3	
A. fumigatus	8000	16,000	16,000	8000	16,000	16,000	13,300

* The results of the triplicate tests with each organism as the reciprocal of the dilution that showed inhibition/killing

** NI = not inhibited

EXAMPLE 4: In-vitro effectiveness of different terpene formulations.

This example shows the amount and types of terpenes from six different terpene formulations (table 2). In the microbiological study seven microorganisms including *Escherichia coli, Salmonella typhimurium, Pasteurella mirabilis, Pseudomona aeruginosa, Staphylococcus* aureus, *Candida albicans*, and *Aspergillus fumigatus* were utilized. These microorganisms were selected in view that they are commonly present in infections and contaminate animal products utilized for human consumption. Each organism, except *A. fumigatus*, was grown overnight at 35-37 °C in tryptone broth. *A. fumigatus* was grown for 48 hours. Each organism was adjusted to approximately 10^5 organisms/ml with sterile saline. Each terpene formulation was diluted to 1:500, 1:1000, 1:2000, 1:4000, 1:8000 and 1:16000 in broth and/or saline. Each terpene formulation dilution was added to sterile tubes in 5-ml amounts and 5 ml of the test organism was added to each series and incubated for 1 hour. There were three replicates of each series of dilutions for each test organism. After incubation half a ml of each tube was plated onto blood agar and incubated 18-24 hours at 35-37 °C. The *A. fumigatus* test series was incubated for 72 hours at 25 °C. The minimum inhibitory concentration for each test organism was determined as the highest dilution that completely inhibits the organism growth. The microbiological results are presented on table 3.

Table 2: Terpene formulation used for antimicrobial testing

	Formulas (%)					
Terpenes	**A**	**B**	**C**	**D**	**E**	**F**
Citral		15			20	70
Carvone			55	55	35	10
Eugenol			35		40	10
B-ionone	30	80	10	40		
Liposome	70					
Tween-80		5	5	5	5	10

Table 3: Effect of different terpene formulations on microorganism growth

Dilution at which microorganism growth was inhibited						
Organism	Formulas					
	A	**B**	**C**	**D**	**E**	**F**
E. coli	NI	NI	NI	NI	2000	1000
P. aeruginosa	NI	NI	NI	NI	2000	NI
P. mirabilis	NI	NI	NI	NI	1000	1000
S. typhimurium	NI	NI	NI	NI	2000	500
S. aureus	NI	4000	1000	4000	2000	1000
C. albicans	NI	1000	2000	2000	2000	1000
A. fumigatus	NI	NI	NI	NI	500	13300
The results are expressed as the reciprocal of the dilution that showed biocidal effect. NI not inhibited						

EXAMPLE 4: In in-vitro effectiveness of terpenes against other fungal microorganisms.

Two terpene formulations were tested against *Sclerotinta homeocarpa, Rhizoctonia solani and Colletotrichum graminicola.* Potato dextrose agar media was amended with each terpene formulation to make a 5000 ppm final concentration of each. For each pathogen, a 5mm-diameter agar plug containing fungal micelia was transferred to each of 5 plates for both terpene formulation and control. All plates were parafilmed and incubated at 25°C. The diameter of fungal colony growth was measured (mm) and recorded. When the control plates were full, measurements were stopped. Colony area was calculated using πr^2, where r is the radius of the colony.

Table 4: Effect of terpenes on fungal growth (area mm^2)

Treatment	*S. homeocarpa*		*R. solani*		*C. graminicola*	
	Day 1	Day 2	Day 1	Day 2	Day 2	Day 7
Formula "A"	0	0	0	0	0	0
Formula "B"	0	0	0	0	0	0

Control	209.0	2023.2	162.3	1976.6	136.7	2023.2

EXAMPLE 6: In vitro effectiveness of single or combination of terpenes against E. coli.

The objective of this example was to determine a terpene mixture that could have an optimal biocidal effect. E. coli strain AW574 was grown in tryptone broth to an exponential growth phase (O.D. between 0.4 and 1.0 at 590 nm). One tenth of this growth was inoculated to 10 ml of tryptone broth following by the addition of individual terpenes or as indicated on table 2; then incubated for 24 hours at 35-37 °C and the O.D. determined in each tube. The concentration of terpenes was 1 or 2 uMol. Each treatment was repeated in triplicate. The results are expressed as percentage bacterial growth as compared to the control treatment. It is observed that the combination of terpenes gives better biocidal effect than single terpenes, with geraniol and carvone better than b-ionone.

Table 5: Effect of single terpene or their combination against on E. coli growth

uMol terpenes			%
B-ionone	Carvone	Geraniol	growth
0	0	0	100.00
2	0	0	84.00
0	2	0	63.00
0	0	2	54.00
1	1	1	41.00
1	2	1	31.10
1	1	2	14.80
1	2	2	15.90
2	1	1	48.60
2	2	1	44.30
2	1	2	30.20
2	2	2	1.50

EXAMPLE 7: Nasal Spray

This example shows a bioactive terpene formulation containing 50% carvone, 30% eugenol, 10% eucalyptus oil and 10% tween-80. The solution was prepared by mixing first the terpenes and then adding tween-80. This mixture was diluted in a standard .9%saline After the solution was agitated it was stored in an off-the-shelf nasal sprayer. In this formulation eugenol is acting as antimicrobial and anesthetic, the eucalyptus oil dilates nasal passages and carvone is also an antimicrobial. This essential oil formulation is effective against bacteria and fungi that may be present in the respiratory system. A preliminary study showed that at 2000 ppm the formulation produced slight irritation. Reducing the concentration to 1000 ppm eliminated this problem.

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ABSTRACT OF THE INVENTION

Prevention and treatment of respiratory infections by the inhalation of a solution containing a single bioactive terpene, a bioactive terpene mixture or a liposome-terpene(s) composition before or after the onset of the infection.

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ACCESS ACKNOWLEDGMENT
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